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L4: Entry 3 of 42

File: PGPB

Jul 22, 2004

DOCUMENT-IDENTIFIER: US 20040142343 A1

TITLE: *Helicobacter bizzozeronii* urease genes and their uses in diagnostic and treatment methodsAbstract Paragraph:

The present invention relates to an isolated nucleic acid molecule conferring on *Helicobacter bizzozeronii* an ability to produce urease. The present invention also relates to the isolation of two urease structural genes, *ureA* and *ureB*, and five urease accessory genes, *ureE*, *ureF*, *ureG*, *ureH*, and *ureI*, of *H. bizzozeronii*. Also disclosed are deduced protein and polypeptide sequences of the nucleic acid molecules and genes of the present invention. Detection and treatment methods relating to *H. bizzozeronii* are also disclosed.

Summary of Invention Paragraph:

[0008] Two of the urease structural subunit genes, *ureA* and *ureB*, and five accessory genes were identified in *H. pylori* and *H. hepaticus* (Beckwith et al., "Cloning, Expression, and Catalytic Activity of *H. hepaticus* Urease," *Infect. Immun.* 69:5914-5920 (2001); Mobley et al., "Molecular Biology of Microbial Ureases," *Microbiol. Rev.* 59:451-480 (1995); Olson et al., "Requirement of Nickel Metabolism Proteins HypA and HypB for Full Activity of Both Hydrogenase and Urease in *Helicobacter pylori*," *Mol. Microbiol.* 39:176-182 (2001); and van Vliet et al., "Nickel-Responsive Induction of Urease Expression in *Helicobacter pylori* is Mediated at the Transcriptional Level," *Infect. Immun.* 69:4891-4897 (2001)). The urease structural subunit genes, *ureA* and *ureB*, of *H. felis*, *H. heilmannii*, *H. mustelae*, and *H. hepaticus* have been cloned and sequenced (Beckwith et al., "Cloning, Expression, and Catalytic Activity of *H. hepaticus* Urease," *Infect. Immun.* 69:5914-5920 (2001); Ferrero et al., "Cloning, Expression and Sequencing of *Helicobacter felis* Urease Genes," *Mol. Microbiol.* 9:323-333 (1993); Labigne et al., "Shuttle Cloning and Nucleotide Sequences of *Helicobacter pylori* Genes Responsible for Urease Activity," *J. Bacteriol.* 173:1920-1931 (1991); Solnick et al., "Construction and Characterization of an Isogenic Urease-Negative Mutant of *Helicobacter mustelae*," *Infect. Immun.* 63:3718-3721 (1995)). The accessory genes are responsible for incorporation of nickel ions into the *UreB* protein and activation of the enzyme (Mobley et al., "Molecular Biology of Microbial Ureases," *Microbiol. Rev.* 59:451-480 (1995); and Olson et al., "Requirement of Nickel Metabolism Proteins HypA and HypB for Full Activity of Both Hydrogenase and Urease in *Helicobacter pylori*," *Mol. Microbiol.* 39:176-182 (2001)). *UreI* is essential for bacterial survival in a low pH environment (Rektorschek et al., "Acid Resistance of *Helicobacter pylori* Depends on the *UreI* Membrane Protein and an Inner Membrane Proton Barrier," *Mol. Microbiol.* 36:141-152 (2000); and Skouloubris et al., "The *Helicobacter pylori* *UreI* Protein is Not Involved in Urease Activity but is Essential for Bacterial Survival In Vivo," *Infect. Immun.* 66:4517-4521 (1998)).

Summary of Invention Paragraph:

[0017] The present invention also relates to an isolated nucleic acid molecule from a *Helicobacter bizzozeronii* urease gene cluster, where the nucleic acid molecule is one of the following genes located in SEQ ID NO: 1: *ureA*; *ureB*; *ureE*; *ureF*; *ureG*; *ureH*; and *ureI*.

Brief Description of Drawings Paragraph:

[0023] FIG. 1 shows the restriction enzyme maps of the *Helicobacter bizzozeronii*

urease clones. The urease structural genes ureA and ureB are identified as A and B, respectively. The urease accessory genes ureI, ureE, ureF, ureG, and ureH are identified as I, E, F, G, and H, respectively. Within the restriction maps, the restriction sites are identified as follows: B: BamHI; Ha: HaeI; H: HindIII; N: NdeI; P: PstI; and X: XbaI.

Detail Description Paragraph:

[0032] The present invention also relates to an isolated nucleic acid molecule from a Helicobacter bizzozeronii urease gene cluster. This nucleic acid molecule is one of the following genes located in SEQ ID NO: 1: ureA; ureB; ureE; ureF; ureG; ureH; and ureI.

Detail Description Paragraph:

[0104] The urease gene cluster from Helicobacter bizzozeronii was cloned and sequenced. A genomic library was constructed in a .lambda.-ZAPII vector using TSP5091-digested H. bizzozeronii chromosomal DNA. Four overlapping recombinant bacteriophages carrying the H. bizzozeronii urease genes were identified by using a fragment of H. bizzozeronii ureB as a probe. Sequence analysis of two clones (pHB1 and pHB3) revealed seven open reading frames encoding proteins with predicted masses of 26.5, 60.3, 21.7, 19.5, 28.6, 21.7, and 29.6 kDa representing the structural genes, Urease A and B, and its accessory genes, urease I, E, F, G and H, respectively. In addition, three open reading frames upstream of the ureA gene encoding a putative tRNA transferase, a putative Glucose inhibited division protein B (GidB) and a protein with unknown function were also identified. A clone (pHB5) containing a complete urease gene cluster was constructed. The homologue analysis revealed that UreA polypeptide exhibited 64-90% identity to that of H. heilmannii, H. felis, H. pylori, H. mustelae, and H. hepaticus. UreB polypeptides exhibited 76.8-96% identity to that of H. heilmannii, H. felis, H. pylori, H. mustelae, and H. hepaticus. The UreI, E, F, G and H also showed 44-86% identity to that of H. pylori. Among these accessory genes, UreE had a lowest percentage identity to that of H. pylori.

Detail Description Paragraph:

[0118] TTG codons initiated ureE and ureH ORFs and ATG codons initiated the rest of the ORFs of the urease gene cluster. The intergenic region between ureB and ureI contains about 188 bp. This structure is similar to that of H. pylori (192 bp) but different from H. hepaticus which contains only 9 bp between the ureB and ureI genes. However, the intergenic regions between ureB and ureI DNA sequences of H. pylori and H. bizzozeronii are heterologous. The ureAB sequence was examined for E. coli promoter-like sequences by the homology score method (Mulligan et al., "Analysis of the Occurrence of Promoter-Sites in DNA," Nucl. Acid Res. 12:789-800 (1984), which is hereby incorporated by reference in its entirety). There was a sequence, TAGAAT, similar to the TATAAT consensus promoter sequence (-10 region) and one sequence, TTAACA, similar to the consensus RNA potential ribosome-binding site (-35 region) proximal to ureA (FIGS. 2A-2C).

Detail Description Paragraph:

[0126] UreI from H. bizzozeronii was 77.4% homologous to that of H. pylori. UreI is essential for activation of cytoplasmic urease at low pH (pH<4.0), but is not required for biogenesis of active urease (Rektorschek et al., "Acid Resistance of Helicobacter pylori Depends on the UreI Membrane Protein and an Inner Membrane Proton Barrier," Mol. Microbiol. 36:141-152 (2000); Scott et al., "Expression of the Helicobacter pylori ureI Gene is Required for Acidic pH Activation of Cytoplasmic Urease," Infect. Immun. 68:470-477 (2000); and Skouloubris et al., "The Helicobacter pylori UreI Protein is Not Involved in Urease Activity but is Essential for Bacterial Survival in vivo," Infect. Immun. 66:4517-4521 (1998), which are hereby incorporated by reference in their entirety). UreI has properties similar to those of a highly selective H.sup.+ -gated urea channel (Weeks et al., "A H<sup>+</sup>-Gated Urea Channel: The Link Between Helicobacter pylori Urease and Gastric Colonization," Science 287:482-485 (2000), which is hereby incorporated by

reference in its entirety). Since ureA is an amide and UreI is homologous to other putative amide transporters (Chebrou et al., "Amide Metabolism: A Putative ABC Transporter in Rhodococcus sp. R312," Gene 182:215-218 (1996); Wilson et al., "Identification of Two New Genes in the Pseudomonas aeruginosa Amidase Operon, Encoding an ATPase (AmiB) and a Putative Integral Membrane Protein (AmiS)," J. Biol. Chem. 270:18818-18824 (1995), which are hereby incorporated by reference in their entirety), UreI may be a member of an amidoporin family of transporters (Rektorschek et al., "Acid Resistance of Helicobacter pylori Depends on the UreI Membrane Protein and an Inner Membrane Proton Barrier," Mol. Microbiol. 36:141-152 (2000), which is hereby incorporated by reference in its entirety). The DNA sequence of the ureI promoter of H. bizzozeronii was compared to that of H. pylori. Unlike H. pylori, there is an incomplete inverted repeat sequence upstream from the ureI transcription start point (Akada et al., "Identification of the Urease Operon in Helicobacter pylori and Its Control by mRNA Decay in Response to pH," Mol. Microbiol. 36:1071-1084 (2000), which is hereby incorporated by reference in its entirety). Also, the intergenic nucleotide sequences between ureB and ureI of H. bizzozeronii and H. pylori are 48% homologous.

Detail Description Table CWU:

16TABLE 1 Homology of the Urease Gene Cluster of Helicobacter bizzozeronii With Other Helicobacter Species COMPARISON PERCENT (%) IDENTITY OF: SPECIES UreA UreB UreI UreE UreF UreG UreH H. heilmannii 90.2 96.0 H. felis 82.9 93.7 H. pylori 76.1 88.1 77.4 44.1 65.8 86.4 49.4 H. hepaticus 64.0 76.8 58.2 31.0 52.7 82.9 44.8 H. mustelae 70.4 77.3

CLAIMS:

3. An isolated nucleic acid molecule from a Helicobacter bizzozeronii urease gene cluster, said nucleic acid molecule being selected from the group consisting of ureA, ureB, ureE, ureF, ureG, ureH, and ureI.

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File: DWPI

Oct 8, 1998

DERWENT-ACC-NO: 1998-557106

DERWENT-WEEK: 199847

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TITLE: New Helicobacter aliphatic amidase AmiE polypeptides and their encoding sequences - used in diagnosis, treatment and prevention of Helicobacter sp. infections in humans and animals

INVENTOR: DE REUSE, H; LABIGNE, A ; SKOULOUBRIS, S

PRIORITY-DATA: 1997US-041745P (March 28, 1997), 1998US-0027900 (February 23, 1998)

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## PATENT-FAMILY:

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<input type="checkbox"/>	US 6248551 B1	June 19, 2001		000	A61K039/02

INT-CL (IPC): A61 K 39/02; C12 N 9/00

ABSTRACTED-PUB-NO: US 6248551B

## BASIC-ABSTRACT:

New polypeptide encoded by a Helicobacter polynucleotide exhibiting amidase activity.

Also claimed are:

(1) a purified polynucleotide corresponding to a Helicobacter aliphatic amidase amiE;

(2) a purified polynucleotide selected from the group comprising: (a) part/all of DNA sequence encoding Helicobacter pylori aliphatic amidase AmiE; (b) part/all of 1885 bp DNA sequence given in the specification; (c) a DNA sequence that hybridizes with part/all of sequences as in (a) or (b) under stringent conditions and encodes a polypeptide possessing biological and immunological properties of Helicobacter pylori aliphatic amidase (or a fragment); and (d) an analogue of sequences (a)-(c) resulting from degeneracy of the genetic code;

(3) a purified polypeptide selected from the group comprising: (a) ATTTCCCTGAATACAGCACGCATGGTATCCTGGGGATCCTGTGGGCGATTG; (b) A DNA sequence hybridizing with (a) under the same conditions as (1 c) together with identical properties of the encoded polypeptide; and (c) an analogue of sequence (a) or (b); or (d) a amidase polypeptide from Helicobacter

(4) a monoclonal antibody specific for the polypeptides (or fragments) or pharmaceutical compositions containing them;

(5) a vector containing any of the polynucleotides; and

(6) a prokaryotic/eukaryotic host cell transformed by the vector.

USE - The polypeptides and antibodies form immunogenic, pharmaceutical and therapeutic compositions used in methods for detecting, treating or preventing Helicobacter sp. (particularly H. pylori and H. heilmanii) infections (claimed), especially chronic gastroduodenal disorders like gastritis, dyspepsia and peptic ulcers in man, and porcine gastric ulcers in pigs. They are administered in immunologically/pharmaceutically effective amounts by an oral, intradermal, intramuscular, intravenous or mucosal route to a patient (claimed). A detection kit for a Helicobacter infection comprises any of the polynucleotides with any of the claimed vectors. The polypeptides are used to in a process to degrade acrylamide, acetamide, propionamide and isobutyramide. The polypeptides are also used to screen for active substrates that inhibit Helicobacter sp. amidase activity.

ADVANTAGE - The polynucleotides encoding the aliphatic amidase AmiE polypeptides are the first to be characterized at the molecular level. Methods involving such polypeptides are preferred to urease-based methods because of the presence of urea positive bacteria in porcine gastrointestinal tracts.

ABSTRACTED-PUB-NO:

WO 9844094A

EQUIVALENT-ABSTRACTS:

New polypeptide encoded by a Helicobacter polynucleotide exhibiting amidase activity.

Also claimed are:

(1) a purified polynucleotide corresponding to a Helicobacter aliphatic amidase amiE;

(2) a purified polynucleotide selected from the group comprising: (a) part/all of DNA sequence encoding Helicobacter pylori aliphatic amidase AmiE; (b) part/all of 1885 bp DNA sequence given in the specification; (c) a DNA sequence that hybridizes with part/all of sequences as in (a) or (b) under stringent conditions and encodes a polypeptide possessing biological and immunological properties of Helicobacter pylori aliphatic amidase (or a fragment); and (d) an analogue of sequences (a)-(c) resulting from degeneracy of the genetic code;

(3) a purified polypeptide selected from the group comprising: (a) ATTTCCCTGAATACAGCACGCATGGTATCCTGGGGATCCTGTGGGCGATTTG; (b) A DNA sequence hybridizing with (a) under the same conditions as (1 c) together with identical properties of the encoded polypeptide; and (c) an analogue of sequence (a) or (b); or (d) a amidase polypeptide from Helicobacter

(4) a monoclonal antibody specific for the polypeptides (or fragments) or pharmaceutical compositions containing them;

(5) a vector containing any of the polynucleotides; and

(6) a prokaryotic/eukaryotic host cell transformed by the vector.

USE - The polypeptides and antibodies form immunogenic, pharmaceutical and

therapeutic compositions used in methods for detecting, treating or preventing Helicobacter sp. (particularly H. pylori and H. heilmanii) infections (claimed), especially chronic gastroduodenal disorders like gastritis, dyspepsia and peptic ulcers in man, and porcine gastric ulcers in pigs. They are administered in immunologically/pharmaceutically effective amounts by an oral, intradermal, intramuscular, intravenous or mucosal route to a patient (claimed). A detection kit for a Helicobacter infection comprises any of the polynucleotides with any of the claimed vectors. The polypeptides are used to in a process to degrade acrylamide, acetamide, propionamide and isobutyramide. The polypeptides are also used to screen for active substrates that inhibit Helicobacter sp. amidase activity.

ADVANTAGE - The polynucleotides encoding the aliphatic amidase AmiE polypeptides are the first to be characterized at the molecular level. Methods involving such polypeptides are preferred to urease-based methods because of the presence of urea positive bacteria in porcine gastrointestinal tracts.

ABSTRACTED-PUB-NO: US 6248551B

EQUIVALENT-ABSTRACTS: New polypeptide encoded by a Helicobacter polynucleotide exhibiting amidase activity. Also claimed are: (1) a purified polynucleotide corresponding to a Helicobacter aliphatic amidase amiE; (2) a purified polynucleotide selected from the group comprising: (a) part/all of DNA sequence encoding Helicobacter pylori aliphatic amidase AmiE; (b) part/all of 1885 bp DNA sequence given in the specification; (c) a DNA sequence that hybridizes with part/all of sequences as in (a) or (b) under stringent conditions and encodes a polypeptide possessing biological and immunological properties of Helicobacter pylori aliphatic amidase (or a fragment); and (d) an analogue of sequences (a)-(c) resulting from degeneracy of the genetic code; (3) a purified polypeptide selected from the group comprising: (a)

ATTTCCCTGAATACAGCACGCATGGTATCCTGGGGATCCTGTGGGCGATTG; (b) A DNA sequence hybridizing with (a) under the same conditions as (1 c) together with identical properties of the encoded polypeptide; and (c) an analogue of sequence (a) or (b); or (d) a amidase polypeptide from Helicobacter (4) a monoclonal antibody specific for the polypeptides (or fragments) or pharmaceutical compositions containing them; (5) a vector containing any of the polynucleotides; and (6) a

prokaryotic/eukaryotic host cell transformed by the vector. USE - The polypeptides and antibodies form immunogenic, pharmaceutical and therapeutic compositions used in methods for detecting, treating or preventing Helicobacter sp. (particularly H. pylori and H. heilmanii) infections (claimed), especially chronic gastroduodenal disorders like gastritis, dyspepsia and peptic ulcers in man, and porcine gastric ulcers in pigs. They are administered in immunologically/pharmaceutically effective amounts by an oral, intradermal, intramuscular, intravenous or mucosal route to a patient (claimed). A detection kit for a Helicobacter infection comprises any of the polynucleotides with any of the claimed vectors. The polypeptides are used to in a process to degrade acrylamide, acetamide, propionamide and isobutyramide. The polypeptides are also used to screen for active substrates that inhibit Helicobacter sp. amidase activity. ADVANTAGE - The polynucleotides encoding the aliphatic amidase AmiE polypeptides are the first to be characterized at the molecular level. Methods involving such polypeptides are preferred to urease-based methods because of the presence of urea positive bacteria in porcine gastrointestinal tracts. WO 9844094A

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☐ 42. US 6248551B. New Helicobacter aliphatic amidase AmiE polypeptides and their encoding sequences - used in diagnosis, treatment and prevention of Helicobacter sp. infections in humans and animals. DE REUSE, H, et al. A61K039/02 C12N009/00.

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Terms	Documents
L2 same (pylori or pyloris or pyloridis or pylori hpylori or h-pylori or helicobacter)	42

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First Hit

L4: Entry 1 of 42

File: PGPB

Oct 14, 2004

DOCUMENT-IDENTIFIER: US 20040204493 A1

TITLE: Carbonic anhydrase inhibitors as drugs to eradicate Helicobacter pylori in the mammalian, including human, stomach

Detail Description Paragraph:

[0014] H. pylori, like E. coli, is a neutralophile, an organism that requires a near neutral pH for growth. Because the medium of the mammalian stomach is highly acidic H. pylori has evolved acid resistance mechanisms to combat gastric acidity to to uniquely colonize the stomach. A major adaptation to acid is the constitutive production of large amounts of intra-bacterial urease enzyme (Mobley et al. Molecular biology of microbial ureases. Microbiol Rev 1995;59:451-480). This urease activity is crucial for bacterial colonization and survival in the harsh acidic environment of the stomach. Activation of urease at acidic pH occurs through the opening of the proton-gated urea channel (UreI) that allows rapid entry of urea into the cytoplasm where it hydrolyzed by the intra-bacterial urease. This results in intra-bacterial production of ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>). It was previously thought that the ammonia produced by urea hydrolysis is able to raise the pH of the periplasm allowing normal cellular functions of H. pylori bacteria. However, recent experiments have shown that the presence of carbonic anhydrase activity is also essential for the periplasmic buffering action induced by the acid activation of UreI (Scott et al. Expression of the Helicobacter pylori ureI Gene is Required for Acidic pH Activation of Cytoplasmic Urease. Infec Immun 2000; 68(2):470-477).

Detail Description Paragraph:

[0027] Therefore, it is now understood in accordance with the present invention that it is the combination of urease activation, expression of UreI and the periplasmic carbonic anhydrase that allows gastric colonization by H. pylori. Hence there is more than a 3 log order of magnitude loss of survival of H. pylori in acid, either with genetic removal of UreI or carbonic anhydrase or in the presence of Diamox.RTM.. Thus, targeting the acid biology of this pathogen provides specific H. pylori therapy. It is recognized in accordance with the present invention that within these three targets, a safe drug is only available for carbonic anhydrase.

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L1: Entry 8 of 26

File: USPT

Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908611 A

TITLE: Treatment of viscous mucous-associated diseases

Detailed Description Text (20):

Acetazolamide is commercially available as Diamox (Lederle Laboratories, Wayne, N.J.), and is typically present in the composition at a concentration of from about 10 micromolar ( $\mu\text{M}$ ) to 10 mM, and preferably about 0.1 to 1.0 mM.

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L4: Entry 3 of 8

File: USPT

Nov 21, 2000

US-PAT-NO: 6149908

DOCUMENT-IDENTIFIER: US 6149908 A

TITLE: Use of lactoperoxidase, a peroxide donor and thiocyanate for the manufacture of a medicament for treating Helicobacter pylori infection

DATE-ISSUED: November 21, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Claesson; Carl Olof	Uppsala			SE
Lindewald; Gustaf	Vallentuna			SE

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Semper AB	Stockholm			SE	03

APPL-NO: 09/ 117029 [PALM]

DATE FILED: July 22, 1998

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COUNTRY	APPL-NO	APPL-DATE
SE	9600233	January 23, 1996

## PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/SE97/00098	January 22, 1997	WO97/26908	Jul 31, 1997	Jul 22, 1998	Jul 22, 1998

INT-CL: [07] A61 K 38/44

US-CL-ISSUED: 424/94.4

US-CL-CURRENT: 424/94.4

FIELD-OF-SEARCH: 424/94.4

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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PATENTEE-NAME

US-CL

<input type="checkbox"/>	<u>4320116</u>	March 1982	Bjorck	424/610
<input type="checkbox"/>	<u>4578265</u>	March 1986	Pellico et al.	424/94.4
<input type="checkbox"/>	<u>5336494</u>	August 1994	Pellico	424/94.4
<input type="checkbox"/>	<u>5453284</u>	September 1995	Pellico	424/94.4
<input type="checkbox"/>	<u>5607681</u>	March 1997	Galley et al.	424/405

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 387 227	November 1990	EP	
88/02600	April 1988	WO	

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Dialong Info. Serv., file 5, BIOSIS, Dialog Asccession No. 7195912, Borch et al.--J. J. Food Prot. 52(9). 1989 (abstract).

ART-UNIT: 161

PRIMARY-EXAMINER: Witz; Jean C.

ATTY-AGENT-FIRM: Larson & Taylor PLC

## ABSTRACT:

Use of an antibacterial system comprising lactoperoxidase and a peroxide donor for preparing a preparation for prophylactic or therapeutic treatment "in vivo" of an infection caused by the bacteria *Helicobacter pylori* existing in the stomach, which preparation is completed by the presence of thiocyanate in an antibacterial level, and eventually in the presence of lactoferrin. A daily dose for human treatment is 1.2-1.6 grams of the system taken 3 times a day.

7 Claims, 2 Drawing figures

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